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Research Article

The Schistosome Egg in Transit

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Abstract

The *Schistosoma mansoni* egg develops in the female worm ooctype where the oocyte and surrounding vitelline granules become enclosed by a protective shell. The schisto some egg is then transported through the uterus to emerge from the genital pore of the intravascular worm. It is not known how eggs successfully enter the perivascular space and how they transit the intestinal wall into the stools. That this route is far from fail-safe is evident from the fact that eggs only have a fifty-fifty chance to make it from their intravascular location into the environment. The fate of "failed" eggs, which are swept by the blood stream to form end up in the liver of infected individuals, can be studied by injecting schistosome eggs in an experimental thrombosis model into the mouse caecal vein. Perioval coagulation and repair in the liver was demonstrated by immune histology for fibrin and fibronectin. Trapped eggs caused transient fibrin deposition, suggesting a fibrinolytic process. This was confirmed by the demonstration of perioval plasmin in an infected monkey. Fibronectin deposition indicated an early repair process with extracellular matrix formation. That a similar sequence of events may occur in the intestinal wall was suggested by presence of perioval fibrin and fibronectin. However, successful transit of eggs may depend on several mechanisms. Passage through the vascular endothelium is a decisive moment determining the future of the egg and possibly the kind of host response elicited during the journey through the tissues. Our observations on *in vitro* oviposition suggest that the female schistosome ejects the egg through the vascular endothelium. Thus extravasation of "successful" eggs seems to occur by a mechanism distinct from that of failed eggs. This does not exclude the possibility that tissue degradation associated with fibrinolysis may be involved in successful transit of eggs into the excretions.

INTRODUCTION

A key event in the life cycle of schistosomes is transit of the egg from the intravascular female worm into host excretions. The mature egg, the encapsulated ciliated larva, needs to reach water where bursting of the protective shell liberates the parasite, the miracidium, which sets off to infect the intermediate snail host, where the cercaria infectious for the definitive host eventually are produced.

Our understanding of the requirements for successful transit of eggs produced by intravascular schistosomes is complicated by the fact that the sequence of host reactions to eggs in different tissues is hard to establish as eggs are produced continuously over a long time period. It is difficult to distinguish between eggs in the process of being successfully excreted and those, which will be trapped in host tissues. Also in the intestinal wall, tissue damage following the host granulomatous response is common. Clinically this is evident as intestinal and hepatic disease [1]. Thus success is not granted even if the point of egg transfer from the intravascular *S. mansoni* is in the terminal branches of the portal vasculature in vicinity of the intestine. A rough classification into early, intermediate and late granulomas can be established based on cellular constituentsand accumulation of connective tissue components, notably fibronectin and collagens [2,1].

Interestingly there is evidence suggesting that the host response to parasite antigens [3] and the granulomatous immune response [4-6] i s used by the parasite in the process leading to egg excretion [7]. However, the events leading to successful egg excretion have not been identified.

In histological specimens we can only occasionally tell if an individual egg is going to be excreted or if it will be destroyed. Even if we know that all eggs in the liver of an *S. mansoni* infected individual are "failed" it does not exclude the possibility that some host response may serve the interests of the parasite.

Toxic substances diffusing through the eggshell into the environment apparently directly or indirectly cause transit of the egg trough host tissues. From the hosts´ point of view the protective response aims at walling off toxic parasite components and destroying the intruder. Coagulation can be regarded as a first line of defense [8]. A pathogenmay overcome this barrier by induction of fibrinolysis [9-11], but it is also possible that cells involved in granuloma formation, such as activated macrophages could induce fibrinolysis. Thus macrophage degradation of elastic fibers is the result of cooperation between several proteolytic enzymes and plasmin acting as an activator of macrophage elastase [12].

For success, eggs need to be deposited into the extravascular space in the vicinity of the intestinal wall. This apparently occurs as female schistosomes are attracted to substances resorbed from the intestine into the portal blood and therefore migrate

into the terminal branches of the draining vasculature to deposit their eggs [13,14].

While the mechanisms for extravasation of failed eggs can be understood based on observations on eggs after intravenous injection, we do not know how successful eggs get from the female worm through the endothelial barrier into the perivascular tissue. The possibility that eggs could be actively forced through the vessel wall by the female worm has been regarded as unlikely [15].

Here we consider the mechanisms for egg extravasation and the possibility that perioval coagulation is a key event in successful egg transit.

MATERIALS AND METHODS

Experimental infection

Maintenance of the schistosome life cycle in experimental animals was performed as described previously [16]. In short, mice were infected percutaneously with 150 *S. mansoni* cercaria and sacrificed after eight weeks. Adult worms were recovered for in vitro culture by perfusion. To study perioval granulomas of different developmental stages, both liver and intestinal tissues were snap frozen and 10 micron frozen sections containing parasite eggs prepared for immune histology. For reference purposes, tissues were also fixed in formalin or Bouin´s fixative (see below).

For immune histological staining of tissue granulomas some experiments were performed using anti-human plasmin antibodies and tissues obtained from an experimentally infected squirrel monkey (*Saimiri* spp.), an established model for human schistosomiasis [17]. The primate was infected with800 *S. mansoni* cercaria percutaneously and tissues obtained at autopsy six weeks after infection when schistosome eggs were demonstrated in the stools.

Experimental granulomas

To study the kinetics of the host response during granuloma formation in tissues, isolated *S. mansoni* eggs were injected into the caecal vein essentially as described previously [18,19]. Fifteen BALB/c female mice received 8 000 *S. mansoni* eggs in 0.5 ml saline into the caecal vein. Mice were sacrificed at 5 time intervals: 6h, 24h, 3 d, 7 d and 14d. Frozen sections were prepared from liver tissue and used in immune histological staining for fibrin and fibronectin (see above).

In vitro culture of worms recovered from infected animals

Female and male adult *S. mansoni* worms were isolated by perfusion from mice 8 weeks after infection as described previously [16]. Worms were maintained in RPMI 1640 medium for up to one week as described previously [20].

Video recordings and analysis

Video recordings of adult worms in *in vitro* culture were made using VHS as described earlier [21]. In short the a Leitz (Leica DMRB)research microscope and an Olympus 3D preparation microscope equipped with a Sony CCD-IRIS video camera model SSC-M370CE were used for video recordings of adult schistosomes recovered from mice 8 weeks after infection. The frame rate was 15.63 fps. Frames of digitized video recordings were transformed into pictures in png. Format for further visualization analysis of adult female motility at oviposition *in vitro*.

Histology and electron microscopy

Schistosoma mansoni organisms from infected mice were obtained as described previously [16]. Bouin-fixed tissues from infected mice were obtained 8 weeks after infection for paraffin embedding and histological examination by standard methods. Briefly, for histology, 4-micron sections were stained with haematoxylin and eosin (H&E) or silver impregnation to show reticulin fibers. Stained paraffin sections of tissues from a *S. mansoni* infected mouse were photographed to obtain a digital composite by stitching individual adjacent picture frames seamlessly to form a 2-dimensional panorama for virtual microscopy as described previously [22] (see Webmicroscope at http://demo.webmicroscope.net/research/parasitology).

Transmission electron microscopy (TEM) on ultrathin sections of glutaraldehyde fixed tissue samples embedded in *methacrylate* was performed on a Jeol JEM 100C electron microscope.

Immunohistologyandlectin binding

Immuno histological staining of frozen sections of tissues was performed by indirect immune fluorescence essentially as described previously [23]. Fluorochromes used in indirect immune fluorescence staining experiments were FITC and TRITC having maximum emission spectra of around 520 nm and 600 nm respectively.

Fluorochrome-labeled lectins peanut agglutinin (PNA) and soybean agglutinin (SBA) were used as a markers for parasite glycol conjugates in tissues as described previously [24,23]. Staining for fibrin and fibronectin in mouse tissues was performed on frozen sections by indirect immune fluorescence using species-cross reacting antibodies; FITC rabbit anti-human fibrin antibodies (Dako Immunoglobulins, Denmark) and TRITC goat anti-human fibronectin antibodies (Cooper Biomedical Inc, Malvern, USA) [25-27].

Anti-human fibrin antibodies reacting with fibrinogen fragments D, E, X and Y showing no cross reactivity with fibronectin [28] and antibodies against human plasminogen [29] were used in staining experiments on tissue sections of the infected *Saimiri* monkey. Fluorescence microscopy and imaging was performed as described previously [30].

RESULTS

The first part of the results focuses on morphological findings reflecting host defense against eggs in host tissues. Immuno histochemical localization of schistosome antigens and host components related to “first line of defense”: perioval fibrin, fibronectin and plasmin in *S. mansoni* infected animals and in mice injected intravenously with schistosome eggs. Due to continuous egg production, there is a problem of determining the sequence of events around eggs 7 weeks after the initiation of infection. Thus we studied the host response to eggs trapped in the liver after intravascular injection.

The second part concerns the transfer of eggs from the intravascular to the perivascular space. To explore alternative modes of extravasation, an analysis of egg expulsion from the female worm *in vitro* was done.

Perioval Parasite Glycoconjugates, Fibrin, Fibronectin and Plasminin Liver- and Intestinal Granulomas of Experimentally Infected Animals

The majority of eggs in the liver of 8 weeks infected mice are seen in the center of a granuloma. Occasionally, apparently trapped eggs could be seen inside hepatic vessels. Eggs were surrounded by host cells and amorphous material seen at both light microscopy and ultrastructural levels (Figure 1): intercellular material in HE and stained sectionsand TEM. Whereas liver granulomas were circular in tissue sections and contained a centrally located egg, intestinal granulomas frequently appeared to be larger, more irregular and elongated, frequently containing several eggs located in the center (Figure 1 *D*).

Parasite material could be demonstrated by lectin staining in the immediate perioval region (Figure 2 A). Fibronectin was regularly seen around eggs. Some liver and intestinal granulomas contained both fibrin and fibronectin with extensive co-localization (Figure 2 B, C). Occasionally intestinal granulomas contained fibrin, but lacked fibronectin (Figure 2 D). Similar perioval deposits of fibrin and plasmin were seen also in liver granulomas of experimentally *S. mansoni* infected squirrel monkey.

In the infected mice here was a difference between perioval reactivity in the intestine as compared to the liver of the two host proteins: Fibrin was found in 23% of liver granulomas but only in 3% of intestinal granulomas. The proportion was similar for fibronectin; 77% were positive in liver but only 11% in the intestine.

Demonstration of perioval fibrin and fibronectin in mice injected intravenously with S. mansoni eggs

Eggs trapped in the fine hepatic vasculature after injection

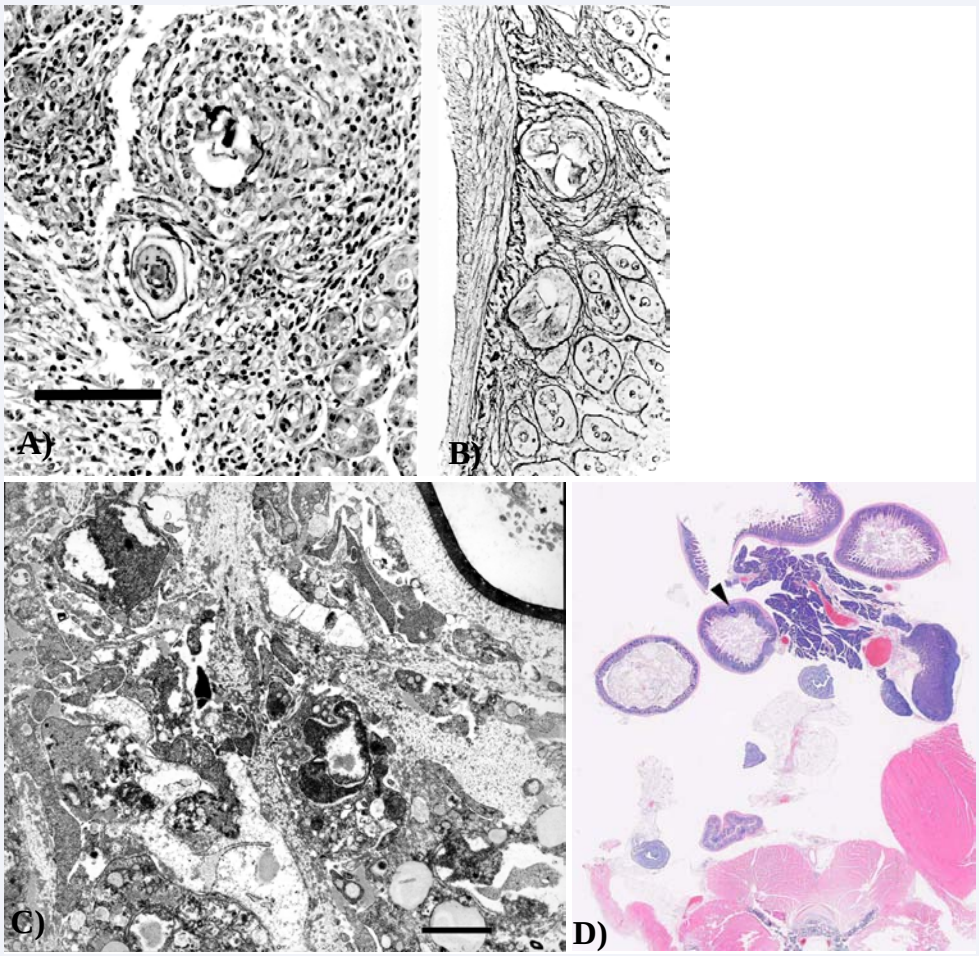

Figure 1 Intercellular matrix in light and electron microscopy of granulomasin Schistosoma mansoni 8 weeks after infection. A B Reticulin fibers seen in granulomas after silver impregnation in liver and intestine. Bar equals 200 microns. C Ultrastructural appearance of intercellular perioval material surrounding the egg shell at upper right corner of picture. Note electron-opaque egg shell matrix with microspines at the outer surface. Cross sections of miracidial cilia surrounded by "hatch fluid" can be seen inside the egg. Bar equals 2 microns. D Tissues of infected mouse can be viewed at desired magnification in the Webmicroscope at http://demo.webmicroscope.net/research/parasitology. Cluster of eggs seen in granuloma in the intestinal wall indicated by arrowhead.

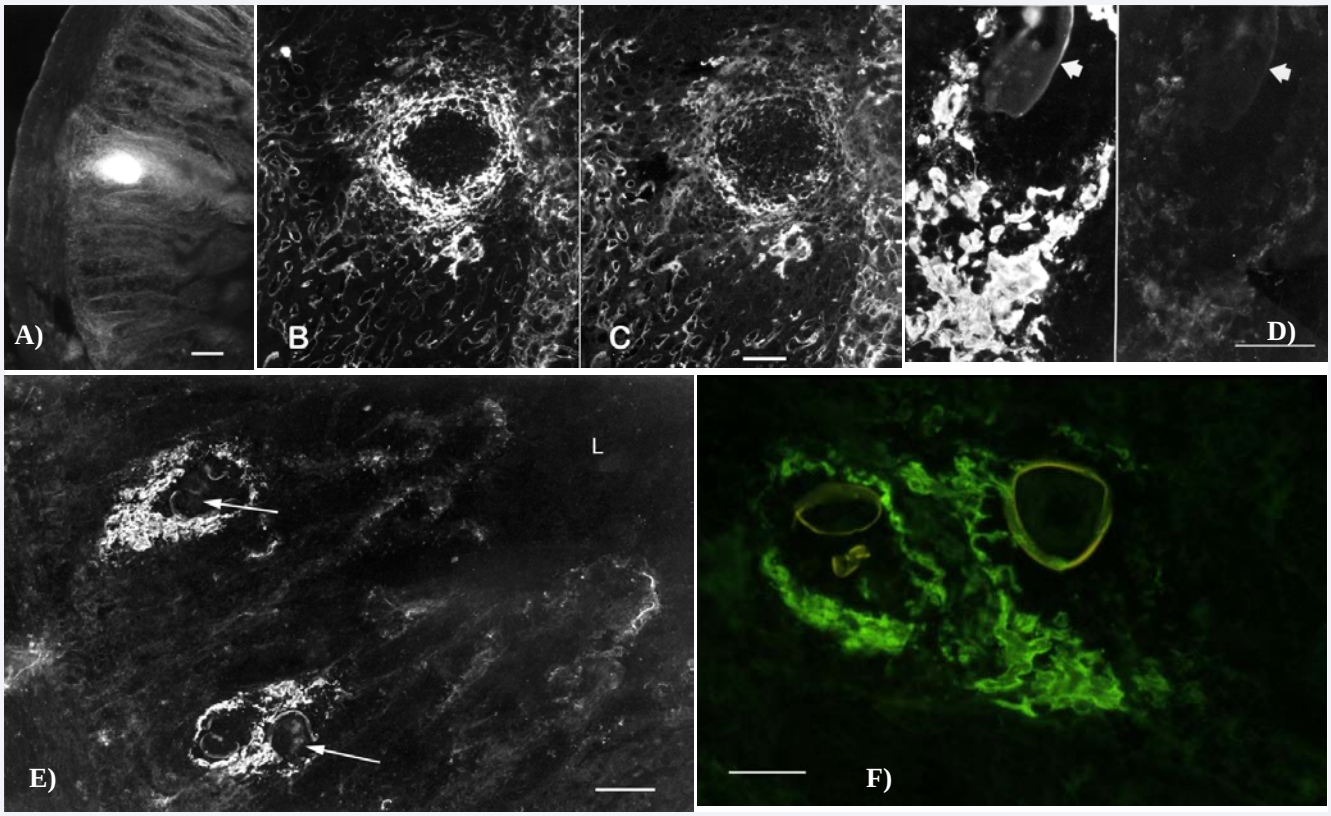

Figure 2 A Intraoval and perioval parasite carbohydrates seen after staining with TRITC labeled soybean agglutinin (SBA) lectin.

B, C Granulomas contained both fibrin and fibronectin with extensive co-localization. D Some intestinal granulomas contained fibrin, but lacked fibronectin.

E Clusters of eggs (arrows) in intestinal wall stained for fibrin Intestinal lumen (L). F Perioval plasmin-reactivity is seen in liver granulomas of experimentally S. mansoni infected squirrel monkey.Bars equal 150 microns.

into the caecal vein is an adequate model for "unsuccessful" eggs, which will not be excreted from the host, but not necessarily an adequate model for extravasation of "successful" eggs. The result of staining for fibrin and fibronectin in the perioval region at various time intervals after intravenous injection of eggs shows fibrin deposition already in the first samples obtained 6 h after injection. Fibrin remains for one week but cannot be seen after two weeks. Maximum fibrin immune reactivity is seen at the firs two time points in specimens obtained 6 h and one day after injection. After one week there was a weak reaction and after two weeks no fibrin could be seen (Figure 3).

Fibronectin is starting to accumulate in regions of fibrin deposits after one day and continues to accumulate over the two-week period.

Egg expulsion by female intravascular schistosome worms at oviposition in vitro

Video recording of egg expulsion shows a backward flexion, a "dorsal nick" of the worm at the excretory orifice just before the egg leaves the worm (video recording at https://youtu.be/LklYLATVK7w). Sequential frames show the progression of an egg in the oviduct, which terminates at the ventral sucker Frames 130 to 270 corresponds to 9 sec recording time. Expulsion of the egg seen in frames 175 to 199 occurs in 1,5 sec (Figure 4).

Note that the egg emerges at the orifice of the oviduct, which is located in close proximity and just caudally to the ventral sucker. Expulsion of the egg occurs rapidly and is accompanied by an almost 180° dorso-flexion lasting for about 10 sec. The contraction has a maximum intensity at frames no. 185-190 and again slightly less intensely in frames around 220. After this, the contraction gradually relaxes and the worm resumes its straight shape, as the egg is seen floating around in the culture medium.

DISCUSSION

Egg extravasation site defines failure or success

Successful transit of *S. mansoni* eggs from intravascular worms into the excretions of the host is a largely unknown process. The results presented here suggest that site and mode of oviposition is a crucial event determining the future of the egg. If extravasation does not take place in the intestinal wall, the egg is swept by the blood stream to cause thrombosis of a vessel in the liver. Extravasationof the egg in the liver leads to its destruction, which is neither in the interest of the parasite nor the host. The parasite life cycle is interrupted and the host suffers because of the tissue damage involved. Both the host and the parasite benefit from rapid transit of eggs through the tissues into the stools. To consider possible mechanisms involvedin the process of egg in transit, we need to identify processes around eggs at various stages of successful and failed transit.

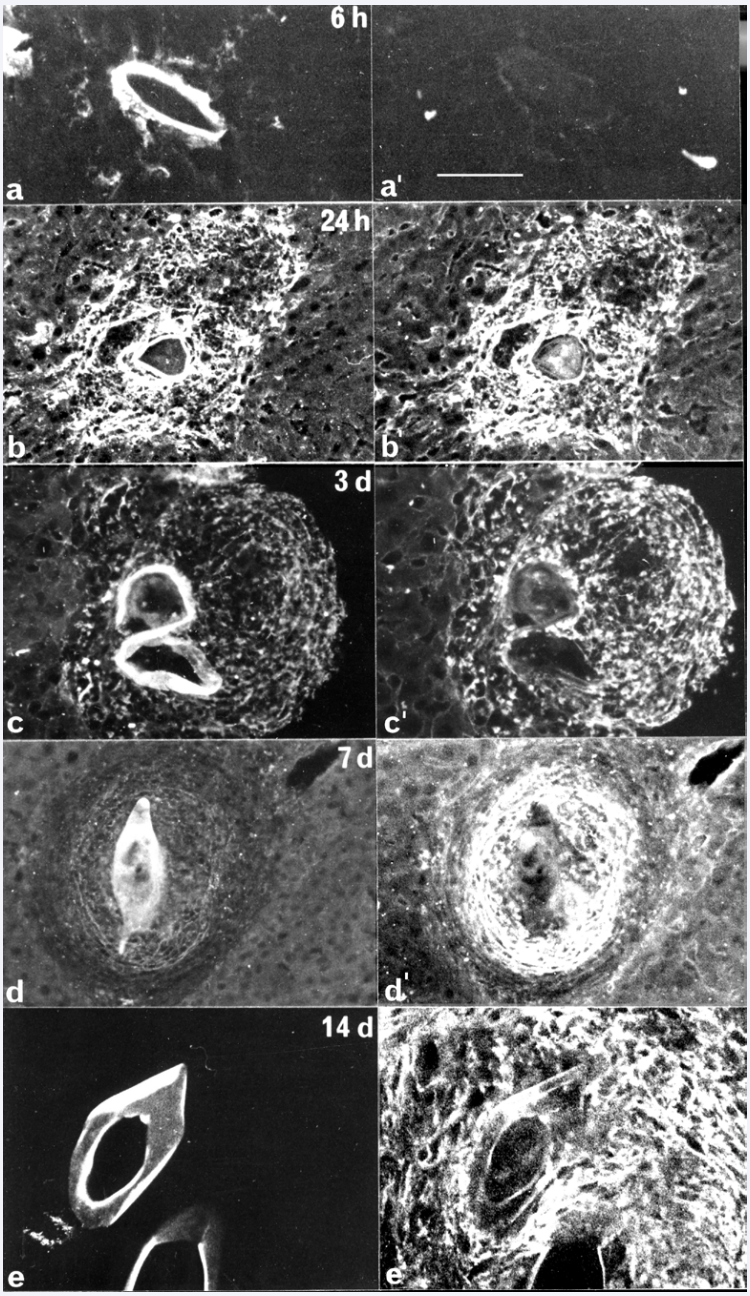

Figure 3 Perioval fibrin and fibronectin in liver of mice at various time intervals after injection of Schistosoma mansoni eggs into the caecal vein. Fibrin deposition is seen already in the first sample, 6 h after injection. Maximum fibrin reactivity is seen in specimens obtained one day after injection suggesting fibrinolysis. After one week the reaction was faint and after two weeks no fibrin could be seen. Fibronectin deposition was seen after 24 h and increased thereafter. Bar equals 150 microns.

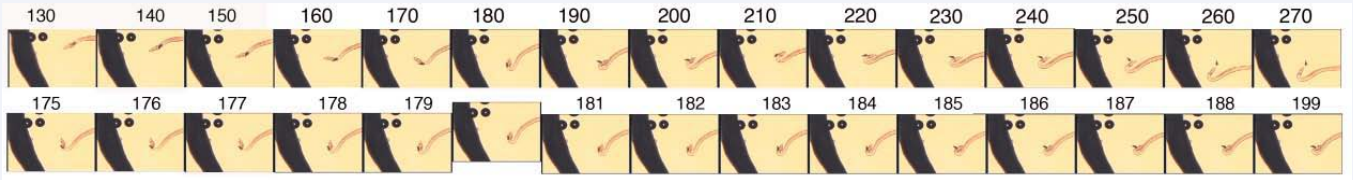

Figure 4 Video frames showing egg emerging from the Schistosoma mansoni ootype into the uterus and then its rapid expulsion through the genital pore. Oviposition is accompanied by an almost 180° dorso-flexion of the female worm lasting for about 10 sec. The contraction has a maximum intensity at frames no. 185-190 and again slightly less intensely, in frames around 220. VHS video recording of female worm cultured in vitro.

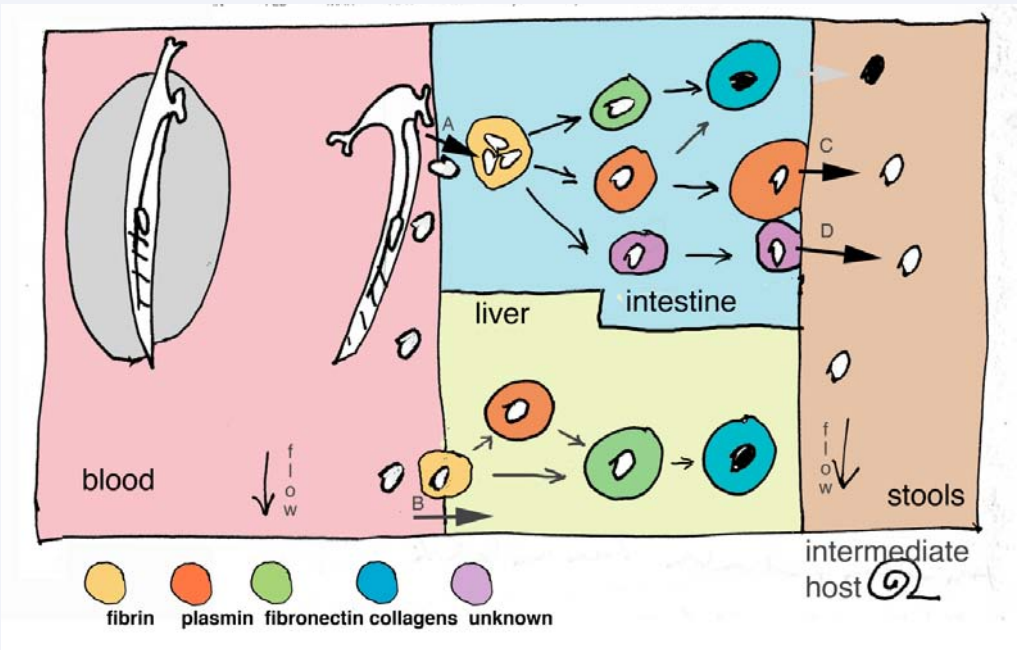

Figure 5 Proposed summary of events leading to excretion of schistosome eggs. To maintain the Schistosoma mansoni life cycle, eggs produced by worms in the vasculature have to enter the perivascular space so that they can transit the tissues of the intestinal wall (A) to end up in the stools. If extravasation fails at the site of oviposition, eggs are swept by the blood stream to end up as thrombi in the fine vessels of the liver (B). Extracellular matrix formation leading to fibrosis can be regarded as a marker for "failed" eggs. However, fibrin deposition and fibrinolysis may occur initially both around "failed" and "successful" eggs. Perioval reactions in the intestinal wall may differ: some eggs may cause granulomas similar to the ones seen in the liver, whereas "successful" eggs may cause perioval tissue destruction not only by plasminogen activation, but also by "cytotoxins" leaking through the egg shell. Local environmental circumstances resulting from mode of extravasation, peristaltic motion, age and aggregation of eggs may contribute to egg transit through the intestinal wall.

The most obvious failure follows egg extravasation at a location such as the liver in the host from where eggs have no chance of leaving the host to encounter an environment harboring the intermediate snail host. This failure of eggs to continue the life cycle of the parasite is associated with a vigorous host response to parasite components and destruction of eggs trapped in tissues.

The events leading to successful transfer of *S. mansoni* eggs begin with extravasation from an intravascular site in the mesenteric vessels draining the intestine. The female schistosome apparently moves against the blood flow towards the intestinal epithelium as a chemotactic response to resorbed nutrients. At oviposition, the egg is thought to get in close contact with the endothelium, but the mechanism for extravasation is not known. Passive transfer of eggs to the perivenular space by a nonspecific migratory response of endothelial cells has been suggested as mechanism for extravasation [31]. No evidence for active pushing of eggs trough the endothelial barrier has been presented 15, We here present an alternative mechanism for successful oviposition by the female schistosomes: The egg is mechanically forced into the perivascular space. Extravasation may occur through egg ejection, associated with a muscle contraction, a "dorsal nick" observed in vitro. This is thought to bring the vessel wall in close contact with the genital pore.

As a consequence of assumed different modes of extravasation, differences in the host response to eggs in the intestinal wall as compared to those in the liver should be expected. As pointed out in this paper, there are some differences, but they could be due to other variables than mode of extravasation. To approach this question, the time sequence of events at the two locations should be compared, but it is experimentally demanding to perform a study on intestinal eggs. Here we present data on the time sequence of perioval coagulation, fibrinolysis and fibronectin deposition after intravenously injected eggs become trapped in the liver vasculature. Possible implications for our understanding of successful egg transit are discussed.

The length of time that eggs remain viable has been calculated to about 3 weeks. Thus they remain in the intestinal wall for less than 3 weeks. Obviously eggs, which are going to be destroied will remain in the tissues for much longer, until they are calcified and surrounded by host connective tissue. Here we focus on early perioval events. A major question is to what extent the observations presented here on perioval reactions in liver following intravenous injection of eggs reflect the perioval response in the intestinal wall. Could the coagulation followed by fibrinolysis explain successful transit of eggs into the stools?

If we compare intestinal wall and liver tissue from mice with schistosomiasis of 8 weeks duration, the perioval response shows some notable differences. Some observations indicate that there are differences between the host response to "successful" eggs and "failed" eggs trapped in the intestinal wall.

Inducers of the host response: Parasite substances leaking through egg shell

The interaction between eggs and endothelial cells may

be an important mechanism for extravasation of failed eggs [31], which is consistent with the observation, that parasite substances leaking through the egg shell into the perioval space [32] can be shown to occur already during the first days after intravenous injection [33]. Four successive stages have been identified based on the morphology of perioval infiltrates and granuloma formation after intravenous injection of schistosome eggs [34,33].

Coagulation and fibrinolysis in relation to parasite components and host cell infiltrates

Fibrin deposits in the liver formed within 24 hours and gradually disappeared after 2 days. This corresponds to stage 1 of Lichtenberg. Trapped eggs showed no perioval monocytes, macrophages or giant cellswithin 24 h. Fibrin deposition around injected eggs occurred before cell infiltrates can be seen periovally. During the second stage "antigen production continued, and there was uptake by host phagocytes. This phase of "antigen sequestration" was followed by rapid antigen destruction ending with the disappearance of capsular deposits as the granuloma reached its peak size" (3rd stage, 16th to 32nd day). "Finally, residual antigen in miracidia and host cells was extinguished, coincident with the healing phase of the granuloma (over 70 days)." [33].

Our findings are consistent with walling of the intruder by coagulation as a first line of defense. This has been noted also seen in tuberculosis granulomas [35].

Perioval fibrin no longer was found periovally seven days after injection. This coincided with the second stage of Lichtenberg, characterized by uptake of antigens by phagocytic cells. The observed plasmin deposits in granulomas from an experimentally infected monkey suggest that activation of plasminogen is involved in fibrin removal. Plasminogen activation apparently is a consequence of macrophage invasion [36] observed early during granuloma formation [33]. However fibrinolysis could also result from enzymatic activity of substances emerging from the egg [37].

Fibronectin and repair

Fibronectin binding apparently occurs in early stages of the repair process and extracellular matrix formation initiated by clotting [38].

For success, *S. mansoni* eggs need to pass the intestinal wall. In the present study we show that eggs in the intestine, like eggs trapped in the liver of infected animals, may evoke a granuloma response involving fibrin and fibronectin deposition

Eggs in transit: success and failure

Morphology of the perioval host response is highly variable ranging from a few leucocytes to massive inflammatory cell infiltrates altering normal hos tissue architecture. In an experimentally infected mouse after 8 weeks granulomas can be seen in the intestinal wall, which extend from the lumen through the muscular layers. Large granulomas in the intestinal wall can be seen to contain several eggs in the center of the granuloma as exemplified in (Figure 1), which is a low magnification screen shot of tissues from an infected mouse which can be examined at various magnifications up to x40 in the Web microscope for Parasitology [22]. Such large granulomas containing several eggs have been described also in the liver [39]. Apparently granulomas containing several central eggs reflect extravasation at the same site. It is likely that accumulation of eggs does potentiate and prolong the effect of substances leaking out through the eggshell. Thereby host tissue destruction and channel formation into the excretions could be achieved. In the intestinal wall some granulomas seemed to contain fibrin, but not fibronectin, as shown in Figure 2D. This suggests that a repair process initiated by fibronectin deposition and followed by collagens may not always take place in the intestinal granulomas. This may be a consequence of accumulation of several eggs, a phenomenon we did not seen in liver granulomas.In the intestinal wall repeated oviposition and extravasation of eggs at the same site may be important: The influx of new eggs may serve to maintain a cytotoxic effect induced by substances leaking through the porous eggshell as newly produced eggs are more efficient in inducing the endothelial response *in vitro* as compared to embryonated eggs recovered from the liver [31]. Possibly, the cytotoxic effect of such newly produced eggs may be enhanced by clustering caused by self-association via the tip of the spine [40]. Such clustering may also contribute to the observed variations in egg output numbers and clustering of eggs within parts of the stool [41] and *in vitro* [42].

Observed large granulomas containing several eggs could represent transit channels containing eggs deposited over a period of time. The higher concentration of cytotoxic egg products of accumulated eggs could modify the sequence of events observed around injected eggs e.g. by preventing the repair process initiated by fibronectin.

The transient presence of perioval fibrin suggested that fibrinolysis occurs in the liver granuloma. This is supported by the observed perioval plasmin deposits in the infected monkey. However, due to lack of available anti-plasmin antibodies at the time, we were unable to follow up our observations systematically by studying plasmin in experimental granulomas. Only a limited number of staining experiments confirming the presence of plasmin and fibrin in granulomas could be done on tissues from an infected *Saimiri* monkey.

Thus our observations on the response to intravenously injected eggs suggesting transient fibrin deposition may reflect also the early events during transit of successful eggs through tissues. However, only a proportion of eggs were surrounded by fibrin. In the intestinal wall they were fewer than those in the liver. A possible explanation is that what we observe reflects both the site and the time spent by an egg in the host environment.

Possibly fibrinolysis around successful eggs is more efficient than fibrinolysis around trapped eggs. This may be a consequence of the shorter time spent in tissues, but also of egg clustering seen in the intestinal wall, but not in liver.

However, we cannot exclude the possibility that fibrin deposition – and presumed subsequent fibrinolysis, is a marker for failed eggs only. Not all eggs in the intestinal wall are successful; some failed eggs will be excreted even if they have been lodged in the intestinal wall. Some are seen as hyalinized,

dead eggs in the intestinal mucosa [1]. It is conceivable that such eggs have evoked a granulomatous response similar to that observed around trapped eggs in the liver. The difference is that in the liver 100% are trapped; unsuccessful eggs and all of them will be destroyed. Can we determine what proportion of eggs will be destroyed in the intestine? If we regard fibronectin accumulation an early marker for trapped eggs with perioval fibrosis, e.g. failed eggs, the answer would be that 77% of all failed eggs are fibronectin positive also in the intestinal wall. The observed 11% fibronectin positive eggs would then suggest that 14% of all eggs in the intestinal wall are trapped. The majority of eggs showed neither perioval fibrin nor fibronectin deposits. Fibronectin was seen in normal connective tissue structures like the liver sinusoids, basement membranes and vessel walls as described earlier [26,25]. The proportion of eggs surrounded by fibrin was only 3% in the intestinal wall as compared to 23% in the liver. The ratio of eggs positive for fibrin to fibronectin was similar in the intestinal wall and the liver. Thus the dynamics of deposition of the two substances appears to be similar; after transient fibrin deposition and as accumulation of fibronectin starts, extracellular matrix proteins like collagens accumulate. The fact that not all liver granulomas were fibronectin positive suggests that fibronectin involved in extracellular matrix formation is replaced by collagens in the process of fibrosis. The 33% of granulomas lacking fibronectin may then be old granulomas seven weeks after infection. This is consistent with the observations by Al Adnani [2].

Preliminary results on staining for plasmin in the perioval region of eggs in the liver and intestine of the *S. mansoni* infected monkey is consistent with the observed transient fibrin deposits around injected eggs and we can conclude that fibrinolysis occurs in perioval granulomas as part of a repair process [43]. In this way plasmin could contribute to egg migration through tissues and excretion as shown for cancer cells [44,43]. That schistosome eggs like tubercle bacilli may induce tissue destruction to gain access to the environment is further suggested by studies of lung lesions caused by intravenously administered eggs [34].

This is consistent with the observed disappearance of fibrin deposits around injected eggs in the mouse model. However, the dynamics of fibrinolysis in association with eggs in transit in the intestine needs to be studied in detail to determine the role of plasminogen activation in egg excretion. We consider that only 3% of eggs in the intestinal wall showed perioval fibrin, the corresponding proportion being 23% in the liver – an eight-fold difference. Does this indicate that fibrinolysis is more efficient periovally in successful eggs? We need to conclude that we do not know what mechanisms are leading to channel formation and excretion of eggs. Also, we do not know if a particular host response benefits the parasite, the host or both [45].

The observed sequential perioval activation of coagulation followed by fibrinolysis and fibronectin is consistent with events following thrombosis [11] and explains what happens to failed eggs but apparently not eggs which are in transit into the intestinal content. The host hemostatic system has been shown to be involved in schistosomiasis [46-49]. The observations are consistent with observations that coagulation is associated with granuloma formation, not only in schistosomiasis, but also in tuberculosis [35].

Apparently perioval fibrin can be removed by plasminogen activation following fibrin deposition but also by fibrinolytic activity of parasite enzymes leaking through the egg shell [37]. It remains to be shown if the egg-derived components like “hepatotoxins” [50,7,51] or other parasite proteases [44] contribute to egg excretion by causing tissue degradation in the perioval area. Such components may affect egg excretion in a variety of ways in the immune host as suggested by the demonstrated immune dependence of egg excretion [7]. It appears that eggs successfully on their way through host tissues to become excreted with the intestinal contents elicit a complex host response, which needs to be studied in such a detail as to reveal both similarities and differences in comparison with the response to eggs trapped in the vasculature.

The challenge is to identify eggs, which will successfully maintain the life cycle of the parasite at an early stage beginning at the transit of eggs through the host vascular endothelium. Currently we only know for sure that eggs, which fail to enter the perivascular space at the site of oviposition, are lost. Thus to understand the complex events leading to egg excretion, details of the host response to perioval parasite components needs to be explored starting from the suggested ejection of eggs through the vessel wall.

CONCLUSION

The transit of schistosome eggs through host tissues into the excretions is an intriguing event in the life cycle of the parasite, which has a 50-50 % success rate. The factors responsible for success involve tissue destruction and channel formation, which seems to depend on both host and parasite components. Based on *in vitro* observations on oviposition by video microscopy and immune histological staining of developing experimental perioval granulomas, a sequence of events is suggested: It seems to implicate active extravasation of the egg by the intravascular female schistosome and host tissue degradation involving fibrinolysis.

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